- Study protocol -

Version 1.1 – 7th of April 2017

Confidential

Title	**Beer after wine versus wine after beer**
Study design	randomized controlled matched-triplet cross-over trial

Information confidentiality

The information enclosed in this test protocol must be treated confidentially. They serve to inform the investigator, the research team, the ethical review committee and the public authorities.

Without consent of the principle investigator of this clinical trail, Dr. Kai O. Hensel, this protocol may not be passed on to third parties.

Index of abbreviations

AHS	Acute Hangover Scale
BAC	Blood Alcohol Concentration
BrAC	Breath Alcohol Concentration
BMI	Body Mass Index

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Synopsis

Title	Beer after wine versus wine after beer
Target population (or indication)	○ male and female participants ○ age: 18 to 60 years ○ provision of written informed consent (see attachment: consent form) ○ positive matched-triplet result ○ positive history of beer and wine consumption
Study design	Randomized controlled matched-triplet cross-over trial
Trial objectives	Primary objective: Comparison of the alcohol-induced hangover severity subject to the order of beer and wine consumption Secondary objective:

	Evaluation of the personal perception regarding reaching of the limit of unfitness to drive (BrAC 0.05%) <u>Tertiary objective:</u> Analysis of subgroups within in the study population regarding their hangover severity ○ female vs. male ○ the effect of BMI, drinking habits, etc. ○ surgeons vs. anaesthesiologists Correlation between laboratory findings and hangover severity.
Study endpoints	<u>Primary endpoint:</u> Rating of the alcohol-induced hangover severity using the AHS ○ scale of 0 to 7 ○ symptoms: thirstiness, fatigue, headache, dizziness, nausea, stomache ache, tachycardia, loff of appetite <u>Secondary endpoint:</u> Difference between the actual measurement of BrAC and the legal limit for unfitness to drive (BrAC 0.05%) <u>Tertiary endpoint:</u> Comparison of the investigated AHS ratings within the subgroups as well as correlation of the laboratory findings with the AHS results
Study sample size	n= 120 probands <u>Study day 1:</u> Study group I: n= 40; first beer, then wine after having reached 0.05% BrAC Study group II: n= 40; first wine, secondly beer after having reached 0.05% BrAC Control group: n= 40; only beer <u>Study day 2:</u> Study group I: n= 40; first wine, then beer after having reached 0.05% BrAC Study group II: n= 40; first wine, then beer after having reached 0.05% BrAC Control group: n= 40; only wine The trial will take place on several days with small manageable groups of volunteers. (see attachment: Flowchart)
Timetable	<u>Trial:</u>

	Recruitment: Summer to fall 2016 Start of data collection: Spring 2017 End of data collection: Summer 2017 Trials will take place on weekends with small manageable groups of volunteers. <u>Regarding the study subjects:</u> Each volunteer will be analysed for several hours including an overnight stay for observation. Each volunteer has to participate in two tests that are at least two weeks apart.
Eligibility criteria	○ Male and female participants ○ Age: 18 to 60 years ○ Provided written informed consent (see attachment: consent form) ○ Positive matched-triplet result ○ Positive history of beer and wine consumption
Exclusion criteria	○ Age: < 18 years ○ Limited legal competence/ability to make legal judgement ○ Chronic alcohol abuse or drug abuse ○ Aversion to beer or wine (or both) ○ Complete alcohol abstinence or intolerance ○ Diseases/conditions that - influence the metabolism of alcohol - may affect the trial outcome as well as - constitute a contraindication for alcohol consumption ▪ Alcoholic liver disease ▪ Viral hepatitis ▪ Hepatocellular carcinoma ▪ Chronic pain ▪ Diabetes mellitus type 2 ▪ Epilepsy ▪ Wernicke encephalopathy, thiamine deficiency ▪ Korsakov syndrome ▪ Gastritis, bariatric surgery ▪ Immunosuppression ▪ Recent history of infection (i.e. respirator, etc.) ○ Pregnancy of breastfeeding ○ Eastern Asian ethnicity [1] - due to prevalence of congenital intolerance to alcohol ○ Use of medications known to interact with alcohol [2] - via cytochrome 2E1 ▪ Paracetamol, Barbiturate, Isoniazid, ▪ Cyclophosphamide, Halothane, ▪ Methadone, Phenylbutazone, Propranolol ▪ Rifampicin, Warfarin, Tolbutamide

	▪ Traquilizer, Vitamin A - via ADH ▪ Cimetidine, Ranitidine, Chlorpromazine ▪ Chloral hydrate - via ALDH ▪ Sulphonylurea, Sulfonamide, ▪ Metronidazole, Griseofulvin, Tolazoline ▪ Procarbazine, antimalarial agents ▪ Chloramphenicol - as well as ▪ Antidiabetics, Antibiotics, Opioids, Nitrates
Course of action	1. Screening and recruitment of volunteers 2. At least 1 week of alcohol abstinence before trial participation 3. History and physical examination as well as reassessment of exclusion criteria on each study day 4. Blood and urine sample acquisition (full blood count, serum parameters, etc.) 5. Administration of a standardized dinner 6. Application of the alcoholic beverages according to randomization a. Documentation of the amounts consumed with Microsoft Excel 7. Repetitive measurements of BrAC of each proband for monitoring a. Documentation of the data with Microsoft Excel 8. Assessment of the secondary endpoint a. Self-evaluation of each proband regarding the legal limit for unfitness to drive (BrAC 0.05%) 9. Switch to the correspondent second alcoholic beverage at 0,05% BrAC (see attachment: Flowchart) 10. Stop of alcohol administration a. Termination desired by the volunteer b. BrAC ≥ 0.11% c. Alcohol-induced symptoms, that require the termination of the intervention or occurrence of exclusion criteria 11. Second taking of a blood and urine samples (blood count, liver enzymes, etc.) 12. Application of individualized amounts of water von (4.5 mL/kg body weight) 13. Re-assessed medical evaluation of the volunteers 14. Monitoring of the probands until reaching BrAC of 0.00% 15. Assessment of the hangover severity using the AHS 16. Data analysis using SPSS and Microsoft Excel 17. Data publication
Interventions and laboratory tests	18. Standardized sampling of blood and urine tests for the inquiry of routine laboratory parameters (full blood count, liver enzymes, etc.) o Frequent Monitoring of the BrAC of the volunteers an AlcoQuant® 6020+ device by EnviteC/Honeywell (Wismar, Germany) o Assessment of the hangover severity using the AHS o Biostatistical analysis of collected data

1. Introduction

1.1 Context of the Study

To this day there is no definition of alcohol-induced hangover (or veisalgia) in biomedical literature. It is described as a complex of symptoms following an evening of heavy drinking that include thirstiness, fatigue, headache nausea and dizziness amongst other things [1,2].

The pathology of the alcohol-induced hangover is poorly understood. Whilst there are multiple attempts to explain its genesis on a molecular level, the findings could not be reproduced or correlated to variating hangover severities. In 2010 Penning et al. wrote a summary of the current state of research in “Current Drug Abuse Reviews”[3]. The authors outline several projects with alterations in enzyme concentrations and blood glucose levels [4] in humans suffering from an alcohol-induced hangover. Furthermore, these cases reported reduced hormone levels and/or impairments of the electrolyte metabolism. However, these studies came to inconsistent results, or correlations with hangover severity were not significant. A different work regarding cytokines by Kim et al. showed significant results correlating hangover severity with alterations in IFN-γ- and IL-12-concentrations [5]. A causality within the discovered association could not be substantiated.

In summary, the biochemical genesis of the alcohol-induced hangover is unexplained to the current state of research. Moreover, the hangover severity seems to be dependent on more than simply the quantity of consumed alcohol. Especially congeners [6] like mineral acid and also the temperature[7] play a well-founded role. The content of congeners in alcoholic beverages differs, and so does it in beer and wine [6]. This might be a reason for the diverse characteristics of the hangover after mixing drinks. Respectively, it is supposable that the consumption of wine, with a higher content of congeners than beer, causes a more severe hangover. Whether or not this is the case, or whether the order of beer and wine consumption shows a measurable dependency is subject to our trial. When drinking beer before wine the individual absorbs more fluid until reaching the 0.05% limit then when drinking wine first, thus a certain dilution might have an assuasive effect on the following hangover symptoms. Given our study design we hypothesize that due to this dilution, drinking first beer then wine leads to a better tolerance and lesser hangover severity then the contrary order.

The „Alcohol Hangover Research Group“ describe in their latest consensus paper the distinct negative socioeconomic and health risks of alcohol-induced hangover [3]. They emphasize that these impairments are severe and largely underestimated compared to other more common diseases. Our trial is designed to answer multiple questions regarding the safe consumption of alcohol and to antagonize the current negligence in hangover research. In particular, we aim to assess the effect of the order of beer and wine consumption concerning the hangover severity on the following day.

1.2 Necessity of Trial Realization

The prevalence of alcohol-induced hangover is alarmingly high. In 1993 an American study showed that 75% of 1104 participants suffered from an aggravating hangover at some point in their life [8]. The same tendency is observable at the place of work as a Norwegian study illustrates [9]. 24.3% of the 526 interviewees stated they went to work with a hangover following a night of excessive alcohol consumption the last year. The economic costs that result from impaired work performance or even

absenteeism at work due to hangover add up to 2.000 $ for each employee a year [10]. Moreover, the risk of injury is significantly higher.

The health risks resulting of frequent alcohol-induced hangover are not to be disregarded. In a Finnish study Kauhanen et al. researched the relationship of hangover-frequency and cardiovascular mortality. They showed that middle aged men with at least one hangover per month have a 2.36-fold higher risk for cardiovascular death then men with fewer hangover incidents [11].
By means of alcohol-induced hangover the risk of accidents rises both at work places as well in sports. In a survey comparing skiers involved in skiing accidents to skiers without accidents 61% of the skiers involved in an accident admitted to the consumption of alcohol in the last 24 hours prior to the accident [12]. Only 19% of the skiers not-involved in an accident had consumed alcohol. Most of the injured participants specified that their last drink was more then 12 hours before the accident. This implicates that the hangover-induced impairment plays a more essential role in the development of an accident than just the acute alcohol intoxication itself.

Alcohol consumptions starts early as an adolescent. A study of the institute for therapy research in Munich, Germany (ITF) surveyed 2034 pupils in grade 9 and 10 [13]. 90.9% admitted to the consumption of alcohol at least once in their life, 70,9% even within the last 30 days. In that process 72.9% reached a state of inebriation. On average, the first consumption of beer and wine happens at the age of 13.4 years. Both genders usually drink beer for the first time (57.7%). Second to beer, girls prefer wine and sparkling wine (51.0%). In the last 30 days 26.7% of the boys drank ≥ 10 times beer and 4,6% ≥ 10 times wine while 11,0% of the girls drank ≥ 10 times beer und 4,0% ≥ 10 times wine.
The data shows remarkably well that alcohol consumption plays a relevant role in our society and starts early as an adolescent, especially the consumption of beer and wine.
In their survey the ITF also gathered information regarding problems that occurred in context of alcohol consumption:

- 7.2% of the adolescents were involved in physical conflicts due to alcohol consumption
- 18.0% were involved in accidents of suffered injuries
    - 1.8% had to be admitted to the ER
- 1.0% was hospitalized due to acute alcohol intoxication
- 6.9% of the adolescents had unprotected sex
- 9.1% of the girls were sexually harassed while consuming alcohol
- 7.0% were driving a car under the influence of alcohol

The number of hospitalizations of 10 to 20-year-olds due to alcohol intoxication increased from 2000 to 2012. This tendency continues for older age groups [14, 15].

Uncontrolled alcohol consumption with resulting hangover constitutes a great socioeconomic problem und furthermore distinctive health risks. Nevertheless, there is only very little research regarding this subject. On October 24th 2010 Google registered 15 million hits using the search term “hangover” whereas there were only 406 scientific publications found on Pubmed during the last 50 years.
Using the keyword “Alcohol” Google found 131million subjects and at least 658,610 publications [16]. For this reason, experts consider that hangover research has to be expanded.

In our trial we aim to use a scientific but realistic approach to illuminate the effects of alcohol after an evening of heavy drinking. The conclusion shall be used to draft a “Recommendation for Safer Alcohol Consumption” that shall be published distinctly and visibly. This way we hope to contribute in making alcohol consumption safer for the society und minimize unnecessary economic costs.

Methodological guidelines from experts in the field [3, 17] suggest to focus scientific research on following items:

a) Pathophysiology of hangover-symptoms
b) Effect of congeners in alcoholic beverages on the alcohol-induced hangover
c) Economic costs due to alcohol-induced hangover
d) Differences between the genders and ages
e) Factors that affect the hangover severity
f) Pharmaceutical products to treat hangover

Our study prioritizes its main issue on the items, b, d and e.

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1.3 Risk-Benefit Assessment

- Primary objective of this study is the assessment of the alcohol-induced hangover severity subject to the order of beer and wine consumption as well as the subjective well-being of each proband. Given our planned realistic study design, our trial will lead to new findings regarding future consumption of alcoholic beverages and help to raise awareness for a healthier and more conservative way of drinking. These findings will be summarized into a "Recommendation for Safer Alcohol Consumption" and published. Many people, especially the youth wonder whether it is risky to drink various types of alcohol alternately. With the completion of this trail we want to accomplish a quantitative reduction in hangover-incidents as well as a qualitative lesser hangover-severity. Subsequently, we hope to reduce hangover-induced economical costs due to impaired work performance and absenteeism.
- Furthermore, we want to promote a more conscious handling of alcohol by sensitizing the self-assessment. During the intervention with alcohol the BrAC of each volunteer will be measured repetitively. By disclosing BrAC measurements to study subjects, the subjective perception of their own wellbeing can be correlated with the objective BrAC assessment, which can improve the subjects' own assessment of drunkenness.
- By publishing the results of our secondary objective, we aim to attract attention on how good or bad the self-assessment of our study population predicts their alcohol-induced unfitness to drive is. Hopefully this way accidents due to alcohol-consumption may be prevented in the future.
- We believe the risk for each volunteer to be minimal since the administration of alcohol will take place under controlled conditions and medical supervision. Furthermore, each proband can terminate the intervention at any time in case of malaise. Alcohol administration will be

stopped at 0.11% BrAC as the protocol dictates or if alcohol-induced symptoms occur that require medical intervention, such as:

- Impaired consciousness, loss of orientation
- Ataxia, gait instability, nystagmus
- Dysarthria
- Malaise (e.g. nausea)
- Changes in blood pressure, tachycardia
- Neurological impairment
- Psychomotor impairment
- Disturbance in attention and concentration
- Prolonged reaction time
- Impairment of the adverse-effects reflexes
- Respiratory impairment
- Illusionary hallucinations

- Prior to the intervention each proband receives a standardized meal to facilitate a better alcohol tolerability (details given below).
- In a collective of 562 alcohol intoxicated patients Frezzottia et al. showed that laboratory parameters indicating that liver function does not become abnormal below BrAC levels of 0.20% [18].
- In the unlikely event of a complication a team of doctors and paramedics will be involved. The volunteers will be under their supervision until BrAC returns to 0% when they may go home.
- Every weekend people consume alcohol in substantial amounts in bars and clubs. Our study design reflects that exactly but places it under controlled conditions and medical supervision. Retrospective studies show that without this supervision measured BrAC would oftentimes peak far beyond the toxic threshold [18-20].
- Many studies regarding the evaluation of alcohol-induced impairments can be found in databases. Especially in recent years a lot of research took place for the better understanding of the effects of alcohol. Main focus of these trials were the investigation of alcohol-induced impairment while driving [21-23] as well as the cognitive impairment [23-25]. In consideration of this trend and the recommendations of the “Hangover Research Group”, we believe that it is ethical and even essential to generate new data through sound scientific research.

2 Trial Objectives

2.1 Primary Objective

Primary objective is the comparison of the alcohol-induced hangover severity subject to the order of beer and wine consumption

2.2 Secondary Objective

Secondary objective is the investigation of the volunteers’ self-assessment regarding the question: „When do you believe you cannot legally operate a car anymore, or in other words, when do you think you have reached the legal limit (0,05% in Germany) for unfitness to drive?“

2.3 Tertiary Objective

Tertiary objective is the comparison of subgroups within the study population regarding the hangover severity

- male vs. female
- effect of BMI, drinking habits, etc.

Furthermore, correlations between laboratory findings and hangover severity will be evaluated.

3 Description of the experiment

3.1 Study Design

This is a prospective clinical randomized controlled matched-triplet cross-over trial with a single investigator comparing adults without alcohol abuse between the age of 18 to 60. Afterwards all collected data will undergo biostatistical analyzation.

3.2 Primary Endpoint

Primary endpoint of this study is the assessment of the alcohol induced hangover severity using the Acute Hangover Scale (AHS) [26]. The AHS is a compound score including the following hangover-associated symptoms: thirstiness, fatigue, headache, dizziness, nausea, stomach ache, tachycardia and loss of appetite. These eight items are rated from 0 to 7 the day after excessive alcohol consumption not before BrAC has returned to zero. Maximum value is therefore 56.

The results will be compared and analyzed between the two study groups and the control group.

3.3 Secondary Endpoint

The secondary endpoint is the difference between the measured BrAC of the volunteer and the legal limit for unfitness to drive of 0,05%.

3.4 Tertiary Endpoint

The tertiary endpoint is the comparison of subgroups within the study population using the AHS. Furthermore, we will correlate urinary and blood laboratory findings collected during the alcohol-induced hangover with the AHS results.

3.5 Study Sample Size

The study aims to include 120 volunteers randomizing them into two study groups and one control group - Study group I: n= 40, Study group II: n= 40 and the control group: n= 40.

3.6 Timetable

Regarding the trial:

- Recruitment: Summer to fall 2016
- Start: Spring 2017
- End: Summer 2017

Trials will take place on weekends with small manageable groups of volunteers.

Regarding the probands:

- Each volunteer will be analysed for several hours including an overnight stay for observation.
- Each volunteer participates in two interventions that are at least two weeks apart.

4 Study Population

4.1 Eligibility Criteria

Only healthy adults with a negative medical history will be included in the study.

- Male and female participants
- Age: 18 to 60 years
- Provided written informed consent (see attachment: consert form)
- Positive matched-triplet result
- Positive history of beer and wine consumption

4.2 Exclusion Criteria

- Age: < 18 years
- Limited legal competence
- Chronic alcohol abuse or drug abuse
- Aversion to beer or wine (or both)
- Complete alcohol abstinence or intolerance
- Indispositions and conditions that
  - influence the metabolism of alcohol
  - may affect the trial outcome as well as
  - result in a contraindication for alcohol consumption

        - Alcoholic liver disease
        - Viral hepatitis
        - Hepatocellular carcinoma
        - Chronic pain
        - Diabetes mellitus type 2
        - Epilepsy
        - Wernicke encephalopathy, thiamine deficiency
        - Korsakov syndrome
        - Gastritis, bariatric surgery
        - Immunosuppression
        - Recent history of infection (i.e. respirator, etc.)
    - Pregnancy of breastfeeding
    - Eastern Asian ethnicity [1]
        - due to prevalence of congenital intolerance to alcohol
    - Use of medications known to interact with alcohol [2]
        - via cytochrome 2E1
            - Paracetamol, Barbiturate, Isoniazid,
            - Cyclophosphamide, Halothane,
            - Methadone, Phenylbutazone, Propranolol
            - Rifampicin, Warfarin, Tolbutamide
            - Traquilizer, Vitamin A
        - via ADH
            - Cimetidine, Ranitidine, Chlorpromazine
            - Chloral hydrate
        - via ALDH
            - Sulphonylurea, Sulfonamide,
            - Metronidazole, Griseofulvin, Tolazoline
            - Procarbazine, antimalarial agents
            - Chloramphenicol
        - as well as
            - Antidiabetics, Antibiotics, Opioids, Nitrates

5 Study process

First, the screening phase takes place: Using an online-survey we will register potential probands acquiring the needed information for the following assessment for eligibility - age, gender, ethnicity, height, weight, drinking habit, drinking preference, occupation, medical preconditions, current medication, pregnancy and history of alcohol and/or drug abuse.

Volunteers who fit the eligibility criteria will be matched into triplets and randomized into the two study groups and the control group. Moreover, the volunteers will receive a verbal and written information about the trial followed by their informed written consent.

Probands are asked to remain sober (no alcohol) one week prior to each intervention. On each intervention day, the volunteers consume food and water in normal amounts, reflective of a typical day as judged by each participant individually. Prior to the intervention, each proband will undergo a physical examination and medical history to evaluate their fitness for trial participation and to re-evaluate in- and exclusion criteria. (see attachment: checklist)

Subsequently, blood and urine samples will be collected of each proband to determine baseline in laboratory parameters. Following parameters will be assessed: blood count, liver enzymes, parameters associated with cholestasis, electrolytes, ketone bodies, pH-level and more. This constitutes another checkpoint to evaluate exclusion criteria. A second blood and urine sample will be collected the next morning after the intervention when BrAC has returned to zero. These laboratory findings will be correlated with the assessed AHS rating.

After these preliminary assessments, all subjects receive a standardized meal according to their gender- and age-specific individual estimated energy requirements, calculated as follows [27]:

- PAL: physical activity level → 1,7 for students
- for women:

energy requirement (kcal/24h)= (10x weight [kg]) + 6,25x hight [cm]) – 5x age) – 161

- for men:

energy requirement (kcal/24h)= (10x weight [kg]) + 6,25x height [cm]) – 5x age) + 5

  - the dinner accounts for about 25% of the daily calorie requirement. Therefore, we divide by 4.
- e.g.: 24-year-old men, student, 75kg, 183cm

energy requirement (kcal/24) = ((10x 75kg + 6,25x 183cm – 5x 24 + 5) x1,7) / 4
= 756 kcal

Due to this precaution potential variables that might directly affect the tolerance of alcohol are standardized and comparability of the AHS scores is achieved. Furthermore, the risk of acute gastritis is minimized.

Next, the administration of the alcoholic beverages may begin in accordance with the randomization of each proband. (see attachment: flow chart)

- Study group I: n= 40; first beer, then wine after having reached a BrAC of 0.05%
- Study group II: n= 40; first wine, then beer after having reached a BrAC of 0.05%
- Control group: n= 40; only beer (or only wine)

Alcohol administration will continue until

- termination is prompted by the proband (personal desire) **or**
- a measured BrAC of ≥ 0,11% **or**
- alcohol-induced symptoms that require a medical intervention

In preparation for the intervention, the Widmark-formula is used to calculate the individual amounts of alcohol each proband needs to reach the 0.11% BrAC.

- BAC (per mill, ‰) = (alcohol content (Gramm) x resorption deficit) / (weight (kg) x reduction factor) – elimination rate x hours drinking(h)
- alcohol content(g) = volume (ml) x (vol.% / 100) x 0,8g/ml
- resorption deficit: 0,9
- elimination rate: 0,15‰/h [28]
- reduction factor: male = 0,7 , female = 0,6
- e.g.: a man of 75kg consumes 4 beer (330mL, 5% Alc.) over the course of 4 hours:
  ((4 x 330ml x (5%/100) x 0,8g/ml) x 0,9) / (75kg x 0,7) – 0,1‰ x 4h
  = (52,8g x 0,9) / (75kg x 0,7) – 0,1‰ x 4h
  = 0,51‰

The BrAC of each proband is measured repetitively and documented using an AlcoQuant® 6020+ device by EnviteC/Honeywell (Wismar, Germany). In order to assess the secondary study objective volunteers are asked to approach their study attendant the moment they believe to have reached the legal limit for unfitness to drive (0,05% BrAC). At this point their BrAC is measured and documented. Furthermore, the difference between the measurement and 0.05% is be calculated.

Upon termination of the alcohol application, all participants receive an individualized amount of refrigerated drinking water (6 ml/kg body weight) to be consumed prior to going to sleep. The volunteers have to refrain from any further preventive measures that might mitigate their hangover – such as painkillers, excessive water consumption, etc.
After the intervention all probands will undergo a second orienting medical assessment before going to bed. During the night they will stay under medical observation. They will be discharged the following day after BrAC has returned to zero.

The assessment of the hangover severity using the AHS takes place as soon as BrAC has returned to zero. The second blood and urine sample will be collected then as well.

There will be multiple study days with small manageable numbers of volunteers. Thereby the study organizers can provide optimal medical supervision and monitoring of the probands during the intervention.

Primary endpoint is the assessment of the alcohol-induced hangover severity the morning following the intervention. For hangover assessment, the well-established and validated Acute Hangover Scale by Rohsenow, Howland et al. [26] is used.

Secondary endpoint of this study is the evaluation of the self-assessment of the volunteers to 0.05% BrAC, as it is the legal limit for unfitness to drive in Germany. We will calculate the difference between 0.05% BrAC and the measured BrAC. This self-assessment can be compared between the two study days, respectively the order of consumption of beer and wine, furthermore, revealing any differences regarding the different alcoholic beverages.

Thirdly, subgroups within the study population will be compared regarding their tolerance to alcohol - e.g. male vs. female, etc.

Pseudonyms will be used to register the collected data in our data base. Afterwards it will be impossible to trace back the data to the volunteers' personal identity. The identifiable data will not be available to third parties.

After thorough analyzation of the collected data, our results will be interpreted and published.

5.1 Potential Complications

In theory, complications might occur during the blood sampling. But due to the experience of the study attendants these complications are of a very low possibility

- formation of haematomas
- trauma of a cutaneaous nerve
- risk of needle-stick injury and infection

During the administration of alcohol only a mild level of intoxication is achieved. That way, a hangover on the following morning is probable whilst minimalizing the risk of acute harm or long-term effects for the proband. Alcohol consumption beyond that state is not advised in this study and will be prevented by the lead investigators. Following risks are to consider during mild alcohol intoxication:

- transient ataxia and dysarthria due to impaired psychomotor and cognitive capabilities
- slightly impaired consciousness such as lightheadedness and disinhibition
- nausea and vomiting (e.g. due to acute gastritis)

In case of any complications a team of doctors, paramedics and study attendants will be present each study day. The volunteers will be under medical supervision until their BrAC has returned to zero and they are discharged.

5.2 Selection of the Alcoholic Beverages

To ensure comparability throughout the trial, all volunteers have to consume the same alcoholic beverages. It is plausible that the AHS is prone to alcoholic beverages of lesser qualities and therefore may be falsified. For that reason, only beer and wine with sufficient quality will be administered during the trial.

The wine will originate from one of the 13 German viticultures with a valid certification and an awarded quality control number, which must be featured on the label. The examination includes a

- Test of maturation and breeding
- Analysis of ingredients
- Sensory analysis

The chosen beer will be brewed in Germany. The quality will be ensured by the German “Regulatory decree of beer brewery”.

This study is reliant on the funding by external sponsors. We plan to approach sponsors supported by a positive vote by the Witten/Herdecke University Ethics Committee. Therefore, we cannot specify the brand of the alcoholic beverages at this point in time. In any case we will compare beer and wine of superior quality.

6 Documentation

The lead investigator is responsible for the correct realization of the trial in accordance with the GCP-guidelines, the AMG and the test protocol as well as the respectable entry of the collected data into CRF/eCRF. All collected data must be transmitted into CRF/eCRF by authorized personal. This implies information of potential participants who were excluded from the trial.
The investigator registers the participation of all probands on a specified identification list. The list may be used to identify the probands at a later time and contains their pseudonym, their name, their date of birth and the dates of their intervention. At the end of the trial the identification list remains at the research center.
Furthermore, it must be ensured that the person responsible for documentation in CRF/eCRF can be identified. A list with signatures and abbreviations of the study attendants allowed to document in CRF/eCRF will be stored in the Test File (ISF) and the Trial Master File.

6.1 Case Report Form

It is the responsibility of the lead investigator that all collected data is entered correctly and thoroughly into a trial-specific databank. Only authorized personal or the lead investigator is allowed to make corrections within the eCRF (electronic Case Report Form) if they are substantiated. Still, if need be the original unaltered data must be available after any corrections. All corrections and altered data must be logged stating the date, time and the name of the registrants.

7 Statistics

7.1 Biometric Test Device

The Acute Hangover Scale is a well-established and validated compound score consisting of eight symptoms associated with alcohol-induced hangover. The eight items will be rated from 0 to 7, consequently the maximum score is 56.

- The answer format uses the 0 to 7 scale of Chapman [29] with the four steps of Roehrs [30]: None (0), Mild (1), Moderate (4) and Incapacitating (7).
- The AHS fits the requirements of our study perfectly due to balance in the amount of detail and practicability. It samples the most common symptoms associated with alcohol-induced hangover and showed good utility in past hangover studies.

For each study day all collected data will be documented in a standard data format. After the intervention the data will be processed, analyzed and graphically displayed as mentioned in 7.4.1.

7.2 Study Sample Size

A priori, the study sample size is specified by a statistical power analysis primarily to compare the difference between the two study groups. Expecting a difference of 14% (= 1 total AHS point) difference between the intervention arms and assuming a significance level of 5% for their

comparison with a minimum statistical power of 80%, an effective sample size of 36 probands is targeted for both the wine-beer-beer-wine and the beer-wine-wine-beer samples. Expecting a drop-out rate of 10% we plan to include 40 probands per group, so a total of n=120.

7.3 Blinding

Blinding is not planned.

7.4 Statistical Methods

7.4.1 Target Parameters

Primary, secondary and tertiary target parameters

The primary and tertiary target parameter is the comparison of the assessed hangover severity using the AHS. The data will be collected and documented the morning after the intervention. SPSS will be used for the following analyzation and graphically data presentation.

The secondary endpoint is the difference between the measured BrAC of the volunteer and the legal limit for unfitness to drive of 0,05%. The data will be collected during the intervention and documented subsequently. SPSS will be used for the following analyzation and graphical data presentation.
Furthermore, we will correlate laboratory findings collected during the alcohol-induced hangover period with the AHS results.

7.3.2 Data Analysis

The collected data will be presented in accordance with the respective study groups and control group using statistical parameters (e.g. mean, standard deviation, median, minimum, maximum).

If statistically feasible, the study groups will be compared using analysis of variance. Data will be presented as boxplots showing median, quartiles, maximum value and minimum value.

8 Ethical, legal and administrative aspects

8.1 Responsibilities of Lead Investigator and Head of the Clinical Trial

The head of the clinical trial (Dr. med. Kai Hensel) takes on responsibility for the instigation, organization and financing of the planned clinical trial. He ensures that the clinical examination is conducted consistent with existing laws and regulations, in accordance with the ICH-GCP-guidelines (1996) and the declaration of Helsinki (1996), as well as the instructions of the GCP-enactment (2004). The investigator accepts the demands of this signed test protocol.

Responsibilities of the lead investigator are as follows:
- *Comprehension and implementation of the research plan*

- *Managing time and capacity for the realization of the clinical trial*
- *Collection and documentation of the data, reporting*
- *Delivery of all data to appropriate authorities for audits and/or inspection*
- *Ensuring the confidential processing of all data by all research assistants involved in the trial*

The respective investigator takes responsibility for the realization of the clinical trial at the research center.

8.2 Vote of the Ethics Committee and Notification of Federal Institution

According to § 40 AMG, before starting a clinical trial a positive vote of the responsible ethics committee and the approval of the responsible federal institutions (BfArM or PEI) is essential.
The application is handed in at the Witten/Herdecke University Ethics Committee, faculty of medicine by Dr. med. Kai Hensel (head of the clinical trial).

8.3 Information regarding probands and informed consent

Before the trial, every proband has to provide written informed consent after a detailed clarification of the intervention by the investigator. The information has to be presented verbally and in writing in a comprehensible way that highlights the meaning and consequences of the clinical trial. The contents of the trial information will be documented.

With obtaining signatures by the proband and the investigator with date and name, written informed consent is valid. A copy will be given to the proband whilst the original document is issued in the trial file.

It is stated explicitly, that no interventions will take place until written informed consent is given lawfully by the proband.

8.4 Privacy Protection and Confidentiality

The collection, transmission, storage and analysis of personal data during this clinical trial will take place in accordance with the legislation (Federal Data Protection Act). A written informed consent of the proband prior to the intervention is a prerequisite for this. As part of the detailed clarification about the trial, the proband will be informed about the following:

1. *All data collected regarding this clinical trial will be documented on paper or electronic data storage mediums, treated confidentially, and transferred to research assistants only using pseudonyms*
2. *The written informed consent and the data processing regarding this clinical trial is irrevocable. The participation in this trial may be terminated by the volunteer at any time without giving reasons with no impending disadvantages. In case of a withdrawal, all data collected until that point in time may be analyzed using pseudonyms.*
3. *All collected data will be included in a data bank using pseudonyms, making any backtracking impossible for a third party. Moreover, data will not be available for a third party.*

9 Amendments of the Test Protocol

The issued test protocol is shall remain unchanged to ensure comparability within the research centers and an adjacent flawless analysis.

In exceptional cases, modifications of the test protocol are possible. Any amendments must be subject to consultation between the investigator and the sponsor. All modifications of the planned intervention must be documented, substantiated and signed by all investigators in charge. Subsequently, the amendments are a valid part of the test protocol. If necessary, the study has to be re-approved by the responsible ethics committee and the appropriate federal institutions (e.g. regarding modifications of medication, etc.). Furthermore, the probands have to be notified and give informed written consent again.

Signatures

The following people agree to the contents of this study protocol and evince this by signing. Any alterations regarding the management of this clinical trial must be made known immediately.

Head of the clinical trial

Dr. Hensel, Kai

_____ *07.04.17* Kai Hensel

Name, First name *Date, signature*

Lead investigator

I hereby certify that I read and understood the study protocol at hand and I accept it in all parts. I pledge that all interventions performed by my research center will take place in accordance with the specifications of this protocol

Dr. Hensel, Kai

_____ *07.04.17* Kai Hensel

Name, First name *Date, signature*

The test protocol at hand was issued in consideration of the criteria of the ICH-GCP.

- Checklist -

Name: **Proband-ID:**

Following criteria has to be inquired on every study day.

	No	Yes
Is the informed written consent of the proband at hand?	☐	☐
Is the proband younger than 18 years?	☐	☐
Do any of the following conditions or diseases apply to the proband?		
- Chronic alcohol abuse or alcohol dependency in the past	☐	☐
- Drug abuse	☐	☐
- Complete alcohol abstinence or intolerance	☐	☐
- Aversion to beer or wine (or both)	☐	☐
Does the participant suffer from any of the following diseases?		
- Alcoholic liver disease	☐	☐
- Acute or chronic viral hepatitis	☐	☐
- Hepatocellular carcinoma (HCC)	☐	☐
- Chronic pain syndrome	☐	☐
- Diabetes mellitus Type 2	☐	☐
- Epilepsy	☐	☐
- Wernicke encephalopathy, thiamine deficiency, Korsakov syndrome	☐	☐
- Acute gastritis	☐	☐
- Immunosuppression	☐	☐
- Bariatric surgery	☐	☐
- recent history of infection	☐	☐
- other pre-existing medical conditions	☐	☐
○ If so, what are they? _____		
Is the participant pregnant or does she breastfeed?	☐	☐
Is the participant of Eastern Asian origin?	☐	☐
Does the participant take pain medication regularly?	☐	☐
Other long-term medications?	☐	☐
○ If so, what are they? _____		
○ Does this result in exclusion? (to be filled out by investigator)	☐	☐

Physical examination:

	No pathology	Diagnostic findings
- General condition	☐	_____
- Nutritional state	☐	_____
- Skin	☐	_____
- Mucous membrane	☐	_____
- Heart	☐	_____
- Lung	☐	_____
- Abdomen	☐	_____
- Lymphatic system	☐	_____
- Musculoskeletal system	☐	_____
- Neurological abnormalities	☐	_____

_____ _____

Name, first name Date, signature

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Beer after wine vs. wine after beer

Dear Mr./Mrs. _____,

hangover is commonly understood as the unpleasant feeling the day after alcohol consumption. Biomedical literature describes the alcohol-induced hangover as a complex of symptoms following an evening of heavy drinking that include thirstiness, fatigue, headache nausea and dizziness amongst other things. Moreover, the hangover severity seems to be dependent on more than simply the quantity of consumed alcohol. Especially congeners like mineral acid and also the temperature play a well-founded role. The well-known traditional myths "Grape or grain but never the twain" and "Beer before wine and you'll feel fine, wine before beer and you'll feel queer" suggest the order of consumption to have an effect on the subjective well-being. However, there is no available scientific data to support or discard these sayings.

The main objective of this study is to assess whether or not these sayings can withstand rigorous testing. To achieve this goal, it is necessary that volunteers consume a relevant amount of beer and/or wine under our strict medical supervision after qualifying for trial participation. The trial is designed to reach blood alcohol concentrations that lead to hangover-associated symptoms the following day. Alcohol administration will continue until

- termination is prompted by the participant (personal desire) **or**
- a measured BrAC of ≥ 0.11% **or**
- alcohol-induced symptoms that require a medical intervention

Study design:

We ask all participants to refrain from any alcohol consumption for one week prior to each study participation. On each intervention day, you may consume food and water in normal amounts, reflective of a typical day. Prior to the intervention, medical history will be obtained, and all participants will undergo a physical examination. We ask to inform us of any medical conditions, pregnancies and current long-term medication in detail to avoid any interactions. In this context we will check critically for any exclusion criteria. Furthermore, blood and urine samples will be obtained.
Subsequently, you will receive a standardized meal according to your gender- and age-specific individual estimated energy requirements.

The administration of alcohol will take place under controlled conditions. To monitor your increasing level of intoxication and to ensure your safety, your breath alcohol concentration will be measured repetitively. In order to evaluate your self-assessment regarding the legal limit for unfitness to drive - the secondary objective - you are asked to contact us as soon as you believe to have reached a BrAC of 0.05%. During the intervention you may be switched from beer to wine (or vica versa) depending on your randomization.

Alcohol will be administered only as long as you feel comfortable. You are encouraged to terminate the intervention if any further consumption of alcohol is displeasing. Once reaching a BrAC of ≥0.11% alcohol administration will be stopped, and you will receive no further alcohol. Upon termination another blood sampling will take place. Subsequently, you will receive an individualized amount of refrigerated drinking water to be consumed prior to going to sleep. You are asked to refrain from any further preventive measures that might mitigate your hangover – such as painkillers, excessive water consumption, etc. All participants will have to remain at the research location to stay under medical supervision during the night until their BrAC has returned to zero.

The assessment of the hangover intensity takes place the following day using a standardized questionnaire.

The collection of data will be conducted on two days with a washout period of at least one week in between. Participation on **both study days** is mandatory for this trial.

The course of events is identical on both study days. Solely the order in which you will receive the alcoholic beverage(s) will differ.

We explicitly point out, that no no-fault insurance coverage has been arranged for this trial.

Potential complications:

In theory, complications might occur during the blood sampling. But due to the experience of the study attendants these complications are of a very low possibility

- formation of haematomas
- trauma of a cutaneaous nerve
- risk of needle-stick injury and infection

During the administration of alcohol only a mild level of intoxication is achieved. That way, a hangover on the following morning is probable whilst minimalizing the risk of acute harm or long-term effects for the proband. Alcohol consumption beyond that state is not advised in this study and will be prevented by the lead investigators. Following risks are to consider during mild alcohol intoxication:

- transient ataxia and dysarthria due to impaired psychomotor and cognitive capabilities
- slightly impaired consciousness such as lightheadedness and disinhibition
- nausea and vomiting (e.g. due to acute gastritis)

Summary:

- Medical history and physical examination to check for trial eligibility and exclusion criteria
- two blood withdrawals (approximately 10 mL in total)
  - first: prior to the intervention; i.e. to detect potentially preexistent unknown organ dysfunction

    - second: after alcohol administration in a further medical evaluation for the safety of the participant
- intake of a standardized meal at the research location
- consumption of the alcoholic beverages according to randomization until
    - termination is prompted by the participant (personal desire) **or**
    - a measured BrAC of ≥ 0.11% **or**
    - alcohol-induced symptoms that require a medical intervention
- medical examination upon termination of the intervention to ensure physical wellbeing and further supervision until complete resolution of the intoxication
- Overnight stay with the other participants until complete resolution of the intoxication
- intake of an individualized amount of refrigerated drinking water after alcohol consumption

In case of any further questions, please contact us - contact information is listed above.

Thank you for supporting this trial.

Information sheet / Consent form / Indemnity

Hereby I, _____, born on _____ in _____, agree, to consume alcohol to a mild level of intoxication under controlled settings and that blood withdrawals may be conducted for the purpose of this research.

I was informed, that health damage is only covered by insurance-protection when caused by careless behavior of the investigators. Any further insurance-protection - no-fault insurance - in form of a clinical trials insurance does not exist for this study.

I confirm, I was thoroughly informed about the entire intervention and all possible related complications and agree to its execution. I obligate myself to stay at the research location until the intervention/supervision is ended officially by the lead investigators the following day. Furthermore, I state to waive all possible liability claims and/or rights to compensation for damages that may occur in the course of this study.

Consent form regarding data protection:

I am aware of the fact that in course of this trial, personal information, especially medical findings, will be assessed, saved pseudonymously and analyzed. The utilization of information regarding my personal health will be conducted in accordance with existing laws and requires the following written informed consent prior to participation in this clinical trial. Thus, without my written informed consent I cannot participate in this clinical trial.

I agree, that personal information, especially medical findings, about me may be assessed and documented on paper as well as electronic databases in the course of this clinical trial.

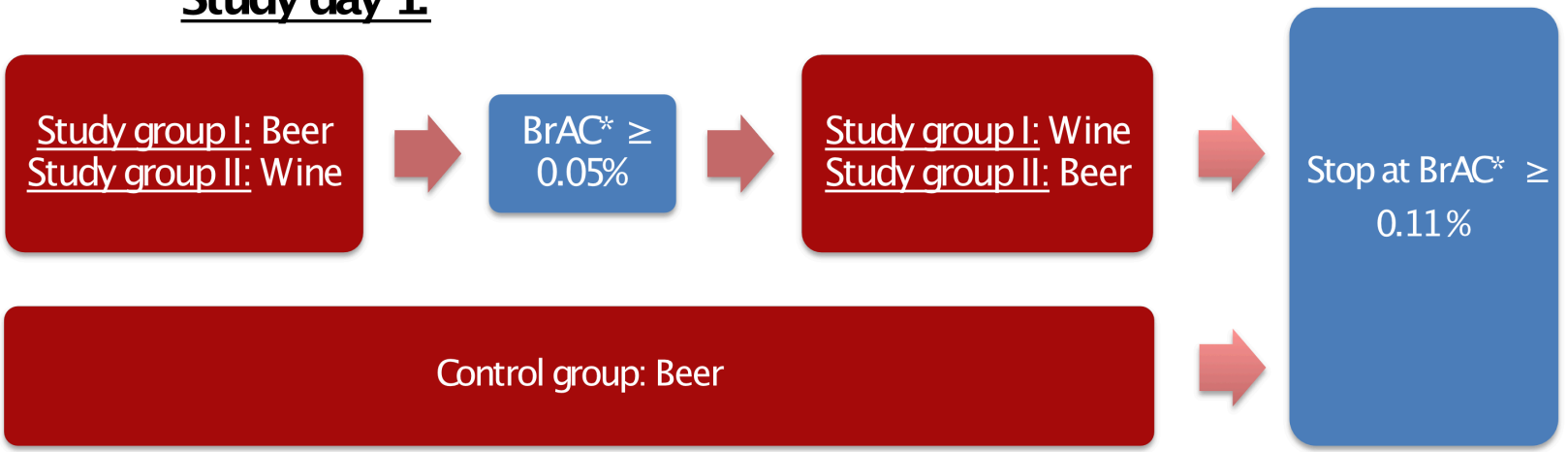
I was informed that I may terminate my participation in this clinical trial at any time. In case of withdrawal, I have the right to demand deletion of all personal data collected until this moment.

Consent:

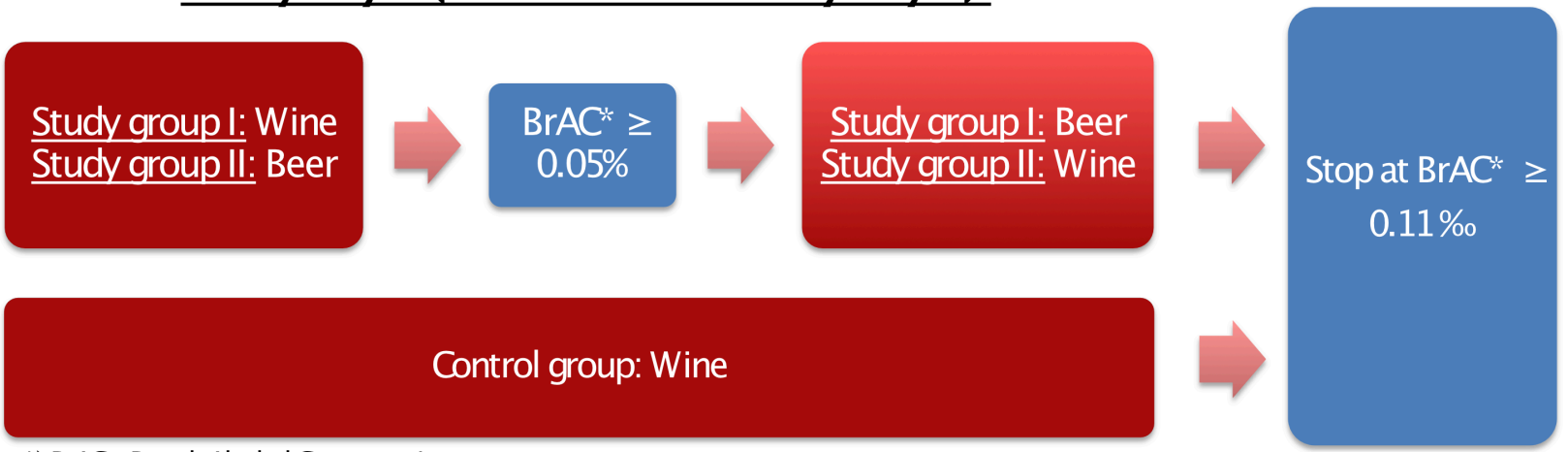
_____	_____	_____
Name (participant)	Date and time	Signature (participant)

Study design: „Beer after wine vs. wine after beer“

Study day 1:



Study day 2 (2 weeks after study day 1):



*) BrAC =Breath Alcohol Concentration